Original Paper

Effect of Resveratrol on the Prevention of Intra-Abdominal Adhesion Formation in a Rat Model

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Background: Intra-abdominal adhesions are a very common complication following abdominal surgery. Our previous studies have demonstrated that the inhibition of inflammation at the sites of peritoneal injury can prevent the formation of intra-abdominal adhesions. Resveratrol is a natural extract with a broad range of anti-inflammatory effects. Therefore, we propose that resveratrol can reduce the formation of intra-abdominal adhesions after surgery. The aim of this study was to investigate the effect of resveratrol on intra-abdominal adhesion prevention in a rat model with surgery-induced peritoneal adhesions. **Materials and Methods:** The cecum wall and its opposite parietal peritoneum were abraded following laparotomy to induce intra-abdominal adhesion formation. Varying doses of resveratrol were administered to the animals. On the eighth day after surgery, the adhesion score was assessed using a visual scoring system. Picrosirius red staining and a hydroxyproline assay were used to assess the amount of collagen deposition in the adhesion tissues. The levels of serum interleukin-6 (IL-6), tumor necrosis factor (TNF- α), and transforming growth factor beta-1 (TGF- β 1) were determined by an enzyme-linked immunosorbent assay (ELISA). Western blotting was performed to determine the protein expression of TGF- β 1, fibrinogen, and α -smooth muscle actin (α -SMA) in rat peritoneal adhesion tissue. Real-time RT-PCR was performed to quantify the mRNA expression of TGF- β 1, fibrinogen, and α -SMA. **Results:** Resveratrol significantly reduced intra-abdominal adhesion formation and fibrin deposition in the rat model. Furthermore, resveratrol significantly reduced the serum levels of IL-6, TNF- α , and TGF- β 1. The protein and mRNA expression of TGF- β 1, fibrinogen, and α -SMA in the rat peritoneum and adhesion tissues were also down-regulated due to resveratrol intervention. **Conclusion:** Resveratrol can effectively prevent the formation of postoperative intra-abdominal adhesions in a rat model. This effect may be related to the suppression of inflammatory cytokine expression in the injured peritoneum by resveratrol. This study suggests that resveratrol may be a new and effective anti-adhesive agent that is worthy of further study and has potential application value.

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Introduction

Postoperative intra-abdominal adhesions are a very common complication of abdominal surgery that may occur in 90 to 95% of patients undergoing abdominal surgery [1]. These unavoidable postoperative adhesions can cause a series of clinical problems such as intestinal obstruction, postoperative abdominal and pelvic pain, female infertility, and difficult access in a subsequent surgery [2, 3]. Intra-abdominal adhesion formation is caused by fibrin exudation and deposition due to inflammation in the injured peritoneum; concurrently, a decreased ability to dissolve fibrin at the injured sites can also lead to adhesion formation [4]. Therefore, at present, the following preventive strategies against postoperative intra-abdominal adhesions should be considered: (1) minimize the surgical trauma; (2) reduce the inflammatory reaction; and (3) reduce the fibrin exudation and promote its absorption. However, thus far, there is no widely accepted method that can safely and effectively prevent intra-abdominal adhesions. Therefore, identifying the ideal method or agent for preventing intra-abdominal adhesions remains a current and urgent need [5].

Resveratrol was extracted from the root of white hellebore for the first time in 1939 by Takaoka [6]. Since then, this natural compound has been identified in grapes, red wine, peanuts, mulberry, pine, berries, and other plants and has gradually attracted researchers' attention. Many studies have demonstrated [7-10] that resveratrol and its analogs or derivatives exhibit good anti-inflammatory activity, antioxidant activity, and protection from heart diseases, neurodegenerative diseases, and metabolic disorders. We conducted a series of experiments [11, 12] to confirm that resveratrol can effectively suppress the body's response to inflammation and reduce inflammatory injury. The results are consistent with those of other studies [13, 14].

During adhesion formation, inflammation is the initial response to peritoneal injury and plays an important role in the formation of intra-abdominal adhesions [15]. We have previously found that the inhibition of inflammation caused by surgical trauma can prevent the occurrence of abdominal adhesions [16]. Our study results were consistent with those of other investigations. To this end, we hypothesized that resveratrol, a natural extract with anti-inflammatory effects, can reduce the occurrence of adhesions. This study was performed to confirm that the administration of resveratrol can prevent adhesion formation in a rat model of surgery-induced peritoneal adhesions. The anti-inflammatory mechanism of resveratrol is associated with the down-regulation of key inflammatory cytokines. Here, we describe a potentially effective agent that may be used to prevent abdominal adhesions in the future.

Materials and Methods

Animal and establishment of intra-abdominal adhesion model

Male Sprague-Dawley rats weighing 200 to 250 g were purchased from the Experimental Animal Center of Xi'an Jiaotong University. These animals were housed at room temperature ($22 \pm 2^\circ\text{C}$) with free access to water and standard rat chow. All animal experiment protocols were approved by the Xi'an Jiaotong University Experimental Animal Ethics Committee.

The animals were anesthetized with the inhalation of methoxyflurane. The abdominal skin was prepared and disinfected with povidone-iodine prior to the procedure. As previously described in the literature [17], a 2- to 3-cm-long lower abdominal midline incision was used to access the abdominal cavity. The pouch-like cecum was located in the right iliac fossa. The cecum wall and its opposite parietal peritoneum were abraded by sterile gauze until the presence of spot bleeding. The abraded area was approximately 2-3 cm² and was exposed to the air for 5 minutes. The bowels were arranged to ensure that the abraded cecum wall was opposite to the abraded peritoneum. In the group with a sham laparotomy, abrasion was not performed on the cecum wall and its opposite parietal peritoneum. In the sodium hyaluronate group, 1 mL of sodium hyaluronate gel was applied to the abraded peritoneum and its surrounding areas. Interrupted 3-0 Vicryl sutures were used to close the peritoneum, the abdominal muscles, and the skin in two layers. Each group included 10 animals.

All animals had no access to water for 4 hours and no access to food for 12 hours after surgery. Three doses (10, 20, or 40 mg/kg) of resveratrol were administered via the penile dorsal vein on the day of surgery for 1 week following surgery.

Adhesion grade and assessment

On the eighth day after surgery, all rats were anesthetized, and an inverted "U"-shaped incision was used to open the abdomen. The magnitude of intra-abdominal adhesions was assessed according to the adhesions grade criteria by Nair et al. [18]. The tenacity of adhesions to the cecum was graded on a scale of 0 to 4: 0 (complete absence of adhesions), 1 (single band of adhesion, between viscera or from viscera to abdominal wall), 2 (two bands, either between viscera or from viscera to abdominal wall), 3 (more than 2 bands, between viscera or viscera to abdominal wall or whole intestines forming a mass without being adherent to abdominal wall), and 4 (viscera directly adherent to abdominal wall, irrespective of number and extent of adhesive bands). The researchers who assess the adhesion grade were independent researchers and were blind to the protocol. The rats were euthanized after the assessment, and samples were collected for the following experiments.

Picrosirius red staining for collagen deposition

The injured cecum wall, parietal peritoneum, and surrounding adhesion tissue were dissected and fixed in 4% paraformaldehyde. After 24 hours of fixation, the tissues were embedded in paraffin, and 4- μ m-thick serial paraffin sections were obtained. Hematoxylin and eosin (HE) staining and picrosirius red staining were performed. Picrosirius red staining of collagen was performed using a 0.1% Sirius red solution (Direct Red 80; Sigma-Aldrich, St. Louis, MO, USA), and nuclei were stained with hematoxylin. The percentage of positive staining area was assessed by ImagePro Plus 5.0 software (Leica Qwin Plus, Leica Microsystem Imaging Solutions Ltd, Cambridge, UK). Eight microscopic fields were randomly selected for measuring the average collagen thickness in the adhesive tissues.

Immunohistochemistry

Immunohistochemical staining was performed using a streptavidin-biotin kit (Maxim, Fuzhou, China) following the manufacturer's instructions. The sections were deparaffinized and rehydrated, incubated with 30 g/L hydrogen peroxide solution at room temperature for 5 minutes and blocked by goat serum; these sections were subsequently incubated with mouse anti-rat antibody α -SMA (sc-53015, 1: 800 dilution; Santa Cruz Biotechnology, Dallas, TX, USA) at 4°C overnight and then incubated with biotinylated rabbit anti-mouse IgG for 20 minutes. Incubation with SABC at 37°C was performed for another 20 minutes. These sections were washed in PBS (pH 7.2) for 5 minutes with four changes. Diaminobenzidine tetrahydrochloride (DAB) was used for visualization, and hematoxylin was used as the counterstain; the sections were dehydrated, mounted, and sealed. An image signal acquisition and analysis system (Leica) was used for image acquisition.

Western blotting

As previously described in the literature [19], western blotting was performed. Total protein was extracted from cell lysates (Mammalian Protein Lysis Buffer, Thermo Scientific Waltham, MA, USA), and protein concentrations were determined using the BCA method. Vertical acrylamide gel electrophoresis and semi-dry transfer to PVDF membranes were performed (Millipore, Billerica, USA). The membrane was blocked with 5% skim milk at room temperature for 1 hour and incubated with the primary antibody dilution buffer at 4°C overnight, which included an anti-TGF- β 1 antibody (sc-146, Santa Cruz Biotechnology, 1: 400 dilution), an anti-fibrinogen antibody (sc-18029, Santa Cruz Biotechnology, 1: 800 dilution), an anti- α -SMA antibody (sc-53015, Santa Cruz Biotechnology, 1: 800 dilution), and an anti- β -actin antibody (sc-47778, Santa Cruz Biotechnology, 1: 1000 dilution). The membrane was washed in PBST buffer for 10 minutes with three changes of fresh buffer and incubated with different dilutions of secondary antibodies in buffer at room temperature for 1 hour. The membrane was washed in PBST buffer for 10 minutes with three changes of fresh buffer. An enhanced chemiluminescence system (Millipore) was used to develop the membrane. Image-Pro Plus 5.0 software (Media Cybernetics, Inc., Rockville, MD, USA) was used to calculate the intensity of the bands.

Real-time RT-PCR

Real-time RT-PCR was performed to determine the messenger RNA (mRNA) levels of TGF- β 1, fibrinogen, α -SMA, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Total RNA was extracted using TRIzol reagent (Invitrogen, CA, USA), and reverse transcription was performed using a PrimeScript RT reagent Kit (TaKaRa, Dalian, China). The real-time experiments were conducted on an iQ5 Multicolor Real-Time PCR Detection System (Bio-Rad, Hercules, CA) using a SYBR Green Real-time PCR Master Mix (TaKaRa). The PCR reactions consisted of 5 seconds at 94°C followed by 40 cycles at 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. The PCR primer sequences were as follows: TGF- β 1, forward: 5'-CAT TGC TGT CCC GTG CAG A-3' and reverse: 5'-AGG TAA CGC CAG GAA TTG TTG CTA-3'; fibrinogen, forward: 5'-GGC AGA TAC TAC TGG GGT GG -3' and reverse: 5'-ATG CTT GGG GGA CTA TTG CTG-3'; α -SMA, forward: 5'-CCG GGA GAA AAT GAC TCA AA-3' and reverse: 5'-GCG TCC AGA GGC ATA GAG AG -3'; GAPDH, forward: 5'-ACC ACA GTC CAT GCC ATC AC -3' and reverse: 5'-TCC ACC ACC CTG TTG CTG TA-3'. The comparative C (T) method was used to quantitate the expression of each target gene using GAPDH as the normalization control [20].

Blood levels of PGE2, IL-6, TNF- α , and TGF- β 1 quantified by ELISA

Blood samples were collected from the inferior vena cava in the animals and centrifuged at 3,000 rpm/minutes for 30 minutes. The serum was stored at -20°C. According to the manufacturer's instructions, the serum concentrations of IL-6, TNF- α , and TGF- β 1 were measured using an ELISA kit (R & D, Minneapolis, MN, USA).

Determination of hydroxyproline content

Hydroxyproline content was measured using a hydroxyproline assay kit (Sigma-Aldrich) according to the manufacturer's instructions. The hydroxyproline content in tissue was expressed as μ g of hydroxyproline/1 g protein.

Data analysis

Quantitative data were expressed as the mean $\pm$ standard deviation or median. Following an analysis of variance, a least significant difference (LSD) test was performed for multiple comparisons in the multiple groups. The Kruskal-Wallis analysis of variance was used to compare the adhesion scores, and the post-hoc Mann-Whitney U test was used for comparisons between groups. Statistical analysis was performed using SPSS 13.0 software. The significance level was set at $P < 0.05$.

Results

Resveratrol reduced abdominal adhesion scores in the rat model

There was no animal death observed, and all animals completed the entire experiment protocol. No wound disruption, wound infection, or intra-abdominal infections were observed in any group. The magnitude of abdominal adhesions between groups was different (Fig. 1). Almost no adhesion formations were observed in the sham laparotomy group. Sheet-like adhesions were observed in the positive control group and were difficult to separate. In the sodium hyaluronate group, the jelly-like sodium hyaluronate gel on the animal cecum surface was completely absorbed; partially injured visceral surfaces had been repaired. Strip-like adhesions were observed between the parietal peritoneum and cecum wound bed or between the omentum. In the animals of the low-dose resveratrol (10 mg/kg) group, there was a low magnitude of adhesion information, and the adhesions appeared loose and easy to separate. The abdominal adhesion formations were barely observed in the animals in the moderate- (20 mg/kg) and high-dose (40 mg/kg) resveratrol group.

The magnitude of intra-abdominal adhesions were scored, and a significant difference was observed ($P < 0.05$) (Fig. 2.A) among the five groups of rats. Compared with the control group or the sodium hyaluronate group, the moderate and high doses of resveratrol reduced postoperative adhesions in rats. The proportions of animals with no abdominal adhesions were analyzed, and resveratrol was found to significantly prevent adhesion formation (Fig. 2.B).

Fig. 1. Resveratrol prevents intra-abdominal adhesion formation in rats (n=10). Representative images of intra-abdominal adhesions in each group on day 8. Laparotomy group (A) animals developed almost no intra-abdominal adhesions. Control group (B) animals developed a large number of extensive thick adhesions that were difficult to separate (black triangle). Only slightly fewer intra-abdominal adhesions occurred in the hyaluronan group (C) animals. By contrast, the low-dose resveratrol group (D), middle-dose resveratrol (20 mg/kg) group (E), and high-dose resveratrol (40 mg/kg) group (F) exhibited fewer intra-abdominal adhesions (black arrow) than did controls.

Fig. 2. Adhesion scores and non-adhesion incidence in each group (n=10). (A) Adhesion scores for macroscopic classification. The resveratrol groups had the lowest adhesion scores (* $P < 0.05$). (B) Non-adhesion incidence in each group. The middle-dose and high-dose resveratrol group exhibited the greatest non-adhesion incidences compared with the control group and the hyaluronan group (* $P < 0.05$, Fisher Exact).

Resveratrol decreased collagen deposition in the injured peritoneum of the rat model

We examined the thickness of abdominal adhesions in the injured peritoneum in rats by picrosirius red staining of collagen fibers. As shown in Fig. 3.A, compared with the control group or the sodium hyaluronate group, the amount of collagen in the rat adhesion tissue significantly decreased in the resveratrol group. Decreased collagen was associated with increasing doses of resveratrol ($P < 0.05$). In addition, the resveratrol intervention significantly inhibited the hydroxyproline content of the adhesion tissue, especially in the moderate- and high-dose resveratrol animal groups ($P < 0.05$) (Fig. 3.B). Thus, our data suggest that resveratrol may decrease collagen deposition during adhesion formation in the rat model.

Fig. 3. Effect of resveratrol on collagen deposition in adhesive or injured peritoneal tissues. (A) Representative images of picrosirius red staining for collagen in tissue sections in each group. The adhesive tissue is located between the two lines drawn in the images. (B) The collagen content in the adhesions was significantly decreased in the middle-dose and high-dose resveratrol group compared with the control group. (C) There were significant decreases in the hydroxyproline levels in the three resveratrol groups compared with the control group (* $P < 0.05$) and hyaluronan group (* $P < 0.05$).

Resveratrol inhibited the degree of fibrosis in the injured peritoneum and/or adhesion tissue in the rat model

The degree of fibrosis of the injured peritoneum and/or adhesion tissue in the rat model was examined by immunohistochemical staining of α -SMA, an activated fibroblast marker. In the sham laparotomy group, the peritoneum was intact, and no positive staining was observed. In the control animal group, a large number of fusiform fibroblasts with positive brown staining were seen in the thick adhesive tissue. In the sodium hyaluronate group, the number of fibroblasts with positive α -SMA expression was slightly less than in the control group. However, in the resveratrol group, especially in the moderate- or high-dose resveratrol group, the degree of fibrosis in the injured peritoneum and/or adhesion tissue was significantly decreased (Fig. 4).

Resveratrol suppressed the increase in PGE₂, an inflammatory mediator induced by peritoneum injury, in a rat model

The same method was used to establish intra-abdominal adhesion models in rats. Vein blood samples were collected 6 hours, 1 day, and 8 days after the surgery, respectively. The PGE₂ level in the serum was assessed using ELISA (Fig. 5). Compared with the sham laparotomy group, the serum level of PGE₂ was significantly higher in the control group. This relatively higher level of PGE₂ was persistent 1 and 8 days after the surgery. Resveratrol at the dosages of 10 mg/kg, 20 mg/kg, or 40 mg/kg can significantly suppress the PGE₂ increase induced by peritoneum injury ($P < 0.05$). This suppression effect was not observed in the sodium hyaluronate group ($P > 0.05$). These results indicated that resveratrol can

Fig. 4. Immunohistochemical analysis of α -SMA in intra-abdominal adhesion tissues in groups that underwent peritoneal and cecal abrasion and the normal peritoneum in the laparotomy group (200x).

Fig. 5. Effect of resveratrol on the serum levels of PGE₂ (compared with the control group * $P < 0.05$).

inhibit the inflammatory response induced by peritoneal injury during the entire process of intra-abdominal adhesion formation.

Resveratrol suppressed the blood levels of TGF- β 1, IL-6, and TNF- α in the rat model

Serum levels of TGF- β 1, IL-6, and TNF- α were analyzed using ELISA at 1 and 8 days after model establishment, respectively. The results revealed that the serum levels of TGF- β 1, IL-6, and TNF- α were significantly higher in the control group than in the sham laparotomy group at both 1 day and 8 days after the operation, suggesting that surgical injury-induced adhesion formation was accompanied by significant inflammatory responses. Thus, resveratrol intervention significantly inhibited the increase in TGF- β 1, IL-6, and TNF- α ($P < 0.05$) (Fig. 6).

Resveratrol inhibited TGF- β 1 protein expression in the injured peritoneum and/or adhesion tissue in the rat model

Western blot analysis revealed that TGF- β 1 expression in the abdominal adhesion tissue was significantly higher in the control group than that in the sham laparotomy group

Fig. 6. Effect of resveratrol on the serum levels of TGF-β1, IL-6, and TNF-α (compared with the control group * $P < 0.05$; compared with the laparotomy group # $P < 0.05$).

($P < 0.05$). Compared with the control group, the sodium hyaluronate group did not exhibit apparent TGF-β1 down-regulation ($P < 0.05$). However, in the resveratrol group (20 mg/kg, 40 mg/kg), the TGF-β1 protein expression in the injured peritoneum and/or adhesion tissue was significantly decreased compared with the control group or the sodium hyaluronate group ($P < 0.05$) (Fig. 7). In addition, we also examined the protein expression of fibrinogen and α-SMA, which exhibited a pattern similar to the TGF-β1 level. The protein expression of fibrinogen ($P < 0.05$) and α-SMA ($P < 0.05$) was suppressed by resveratrol in the injured peritoneum and/or adhesion tissue (Fig. 7).

Resveratrol suppressed the expression of TGF-β1, fibrinogen, and α-SMA mRNA in the injured peritoneum and/or adhesion tissue in the rat models

We used real-time RT-PCR to analyze the mRNA expression of TGF-β1, fibrinogen, and α-SMA in the injured peritoneum and/or adhesion tissue in each group at 8 days after the

Fig. 7. Effect of resveratrol on the expression of TGF-β1, fibrinogen, and α-SMA in adhesive or injured peritoneal tissues (A). Western blot analysis revealed that resveratrol obviously decreased TGF-β1 (B), fibrinogen (C), and α-SMA (D) protein expression in the injured peritoneum compared with adhesion tissue in the control group (* $P < 0.05$).

surgery. The mRNA levels of TGF-β1, fibrinogen, and α-SMA were significantly higher in the intra-abdominal adhesion tissue in the control group compared with the sham laparotomy group ($P < 0.05$). Compared with the control group, mRNA levels of TGF-β1, fibrinogen, and α-SMA in the sodium hyaluronate group were not significantly decreased ($P > 0.05$). Compared with the control or the sodium hyaluronate groups, mRNA levels of TGF-β1, fibrinogen, and α-SMA in the injured peritoneum and/or adhesion tissue of the resveratrol groups (20 mg/kg, 40 mg/kg) were significantly suppressed ($P < 0.05$, Fig. 8). These results were consistent with the protein expression analysis by Western blot.

Discussion

Abdominal adhesions form between two abraded wound beds of the peritoneal lining. Adhesion formation depends on whether the deposited fiber connection is absorbed or undergoes organization; the mechanism of adhesion formation is closely related to inflammation [21]. This study demonstrated that resveratrol can effectively prevent the formation of postoperative abdominal adhesions in a rat model. Resveratrol intervention inhibited the expression of inflammatory cytokines in the injured peritoneum and adhesion tissue. The prevention of abdominal adhesions may be associated with the anti-inflammatory

Fig. 8. Real-time RT-PCR revealed that resveratrol obviously decreased TGF- β 1 (A), fibrinogen (B), and α -SMA (C) mRNA expression in the injured peritoneum compared with the adhesion tissue in the control group (* $P < 0.05$).

effects of resveratrol. Therefore, the present study suggests that resveratrol may be an effective novel anti-adhesive agent that is worthy of further research and may have potential application value.

Abdominal adhesions are a common complication after abdominal and pelvic surgery [22]. Many factors are related to the formation of adhesions after surgery, including surgical injury, foreign bodies, and tissue ischemia, all of which can cause injury and inflammation of abdominal tissue. Cytokines released by infiltrating inflammatory cells and oxidative stress are considered the trigger mechanism and initial step that leads to the formation of adhesions; the deposition of extracellular matrix causes fibrinolysis imbalance in the late stage and promotes the acquisition of adhesions. Thus, the formation of intra-abdominal adhesions is a more complex process that is involved in cells, inflammatory mediators, and cytokines.

In vitro cell culture [23] fibroblasts originating from the adhesion tissue expressed more inflammatory cytokines (IL-6 and TNF- α) than those of fibroblasts originating from normal tissues. However, hypoxic conditions can significantly increase the levels of IL-6 and TNF- α in fibroblasts originating from normal tissues. This result indicated that anti-inflammatory methods may potentially prevent or reduce the formation of post-operative adhesions. An animal experiment that used CO₂ pneumoperitoneum to reduce intra-abdominal adhesions indicated [24] that the adhesion formation was positively correlated with the infiltration of neutrophils and monocytes into the local injury area and with pressure of oxygen stress. In the rat intra-abdominal adhesion model generated by electrocautery to injure the peritoneum [25], Montalvo-Jave EE and colleagues used an anti-adherence compound consisting of a sodium hyaluronate and carboxymethylcellulose gel to reduce the degree of inflammatory infiltration and the number of adhesion lesions. Additionally, some natural compounds with anti-inflammatory and anti-oxidative actions, such as honokiol [26] and bromelain [27], also have effects on preventing the formation of intra-abdominal adhesions, to a certain extent. Our previous studies [16] also verified that adhesion formation in rat models can be prevented using anti-inflammatory drugs, such as selective COX-2 inhibitors. All these results indicated that inhibiting the inflammatory response and release of inflammatory cytokines induced by injury at the early stage may be an effective strategy to prevent adhesion formation.

At present, cytokines related to abdominal adhesions have been extensively investigated. According to the findings of these studies, IL-6 regulates epithelial cell proliferation and promotes the deposition of inflammatory cells and fiber in the injured sites [23]. TNF- α regulates the activity of various cytokines that can stimulate peritoneal mesothelial cells to increase the synthesis of plasminogen activator inhibitor-1 [28]. TGF- β 1 may play a role

as a bridge between the injury and subsequent inflammation and fibrosis [29]. TGF- β 1 can destroy the balance between the formation and degradation of fibrin after surgery-induced injury to the peritoneum; furthermore, TGF- β 1 can cause extracellular matrix deposition, which provides a basis for adhesion formation [30].

Resveratrol is a very strong biological and natural polyphenol [31, 32]. Resveratrol has recently attracted increasing attention because of its reliable and extensive anti-inflammatory effects [33]. In recent reports [34-36] of related studies of its anti-inflammatory effect, the pharmacological mechanism of resveratrol remained unclear, but the mechanism of its anti-inflammatory action may be related to the inhibition of inflammatory signal transduction pathways. A study of astrocytes and microglia by Lu et al. [34] has demonstrated that resveratrol can inhibit NF- κ B phosphorylation and degradation to suppress the expression of TNF- α , IL-6, and other inflammatory factors induced by bacterial lipopolysaccharide (LPS). Many previous reports have indicated [36-38] that resveratrol can inhibit the inflammation-related p38MAPK and NF- κ B pathways to inhibit the production of inflammatory cytokines, such as COX-2, IL-6, and IL-8, and thereby inhibit the inflammatory cascade response. As a new anti-inflammatory agent, resveratrol has been used extensively in the treatment of many diseases, such as osteoarthritis [39], acute pancreatitis [40], colitis [41], esophagitis [41], acute gouty arthritis [42] and airway inflammation [43]. In the present study, various doses of resveratrol were given to a rat model of postoperative abdominal adhesions, and we found that resveratrol had a significant effect on adhesion prevention. Furthermore, inflammatory factors, such as IL-6, TGF- β 1, and TNF- α , were significantly down-regulated in the injured peritoneal tissue and blood. These results suggest that the effect of resveratrol on the prevention of adhesion formation is associated with local and systemic anti-inflammatory effects. Resveratrol may inhibit the release of inflammatory factors in the initial stage of abdominal adhesion formation induced by abdominal injury, suspending the local "cascade response" and ultimately avoiding extracellular matrix deposition and fibrosis.

Mechanical injury, one of the mechanisms of adhesion development, is not limited to the surgical operation site. Surgical exploration in other sites, intraoperative prolonged exposure, and small foreign bodies are likely to cause adhesions [44]. In the present study, we systemically administered resveratrol via an intravenous route to evenly distribute the agent via the blood circulation, thereby avoiding the drawbacks of a topical gel or barrier material, which can only act on the local site. In addition, another advantage of resveratrol is its relative safety and low incidence of side effects. No apparent side effects have been reported since resveratrol became a commercial supplement [45]. Resveratrol exhibits such a low toxicity that it does not cause side effects even in the bone marrow or the digestive tract, which are highly regenerative tissues. Many abdominal surgeries involve a digestive tract operation [46]; therefore, the use of an agent that does not affect the physiology of the digestive tract poses a great advantage.

Various findings [7, 47, 48] suggest that the optimal concentration of resveratrol for anti-inflammatory action in rat models ranges from 10 to 30 mg/kg. In the past, we also performed several studies [11, 40, 49, 50] to demonstrate that resveratrol at 10 ~ 20 mg/kg can suppress severe acute pancreatitis and reduce inflammatory injury in rats. In our current report, we chose three concentration gradients (10, 20, and 40 mg/kg) of resveratrol for animal administration. Our results show that the effect of 10 mg/kg resveratrol for adhesion prevention is better than that of the sodium hyaluronate gel (a commonly used reagent for adhesion prevention). Moreover, 20 and 40 mg/kg of resveratrol have more obvious anti-adhesive and anti-inflammatory effect than 10 mg/kg, with almost no difference between the 20 and 40 mg/kg dosages.

There are certain limitations to this study. A major problem is that the rat model of abdominal adhesions commonly used by researchers may not truly reflect the pathological mechanism of surgery-induced adhesions in the human body because the current understanding of the mechanism underlying abdominal adhesions has not yet been completely confirmed. In addition, the assessment of the magnitude of abdominal adhesions lacks objective parameters, and adhesion grading depends upon the subjective recognition

of researchers. Indirect methods of assessment, such as adhesion tissue thickness and tissue hydroxyproline determination for collagen deposition [51, 52], were used in this study to support the adhesion grading and reduce the bias. Adhesion formation is a dynamic and complex process that can be induced by any peritoneal injury and involves a series of factors including cells, biochemistry, immunology, and biomechanics. Currently, the understanding of the action of these series of factors in the different stages of adhesion development remains incomplete [53] because it is difficult to investigate adhesiogenic events in patients. Therefore, a further understanding of the pathological mechanism of adhesion formation and methods of prevention and treatment remains critical.

In summary, the present study demonstrated that resveratrol can effectively prevent postoperative intra-abdominal adhesion formation in a rat model. This preventive effect may be associated with the inhibition of the inflammatory reaction by resveratrol at the initial stage of adhesion formation. This investigation suggests that resveratrol has broad prospects and good potential value in intra-abdominal adhesion prevention and also provides additional ideas for the development of a new agent to prevent adhesion formation.

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Disclosure Statement

The authors declare that there is no conflict of interest related to this work.

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